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Fluidised Bed Microencapsulation of Ascorbic Acid: Effectiveness of Protection under Simulated Tropical Storage Conditions

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ABSTRACT

This study investigates the use of microencapsulation by fluidised bed coating for the protection of ascorbic acid during long-term storage under simulated tropical conditions. Microencapsulation materials, loading levels, fluidised bed processing conditions and the results of storage trials were evaluated. Cellets® 350 is a suitable base material, whereas sugar spheres are not suitable due to agglomeration and bed collapse during processing. Sodium alginate provided the best overall performance with low processing losses and high retention of ascorbic acid during storage. The results of this study provide a basis for further research including incorporation of ascorbic acid microcapsules into food matrices using a fluidised bed coating.

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Executive Summary

The provision of fresh food to soldiers is a priority for the Australian Defence Force, however when the use of fresh food or canned equivalents is not practicable, combat ration packs (CRP) are used instead (Department of Defence (DoD), 2008a). CRP may be the sole food source for several weeks, but there is a risk that they may not fully meet the nutritional requirements of soldiers for some nutrients.

A major issue is that the storage and distribution system for CRP provides for a delay of up to three years and six months before consumption (DoD, 2009). During this period, the vitamin levels and sensory acceptability of the food decrease, in some cases dramatically. Additionally, some items in CRP are discarded, a significant problem as some of the nutrients are poorly distributed across the range of components. The adequacy of intake may therefore be significantly affected by the choices of the consumer.

These problems may be ameliorated through the fortification of CRP components. In order to ensure that sufficient fortificant to confer the intended benefit remains at the time of consumption, it is important that added vitamins are protected during processing and storage. One method with potential for protection of fortificants is microencapsulation.

The aims of this study were to investigate the protective properties of microcapsules prepared by fluidised bed coating. This includes consideration of processing characteristics and effectiveness in the protection of ascorbic acid (AA) during storage.

The storage trials were conducted so that samples were stored in a temperature and humidity controlled cabinet under conditions intended to approximate the diurnal variation experienced in northern Australia. The temperature was cycled between 25 and 35 °C, completing one full cycle over a 24-hour period, for up to 24 months. Relative humidity (RH) was controlled at 80%. Microcapsules were analysed using capillary electrophoresis to determine AA and the outer structure was examined by environmental scanning electron microscopy.

The results of this study indicate enhanced storage stability compared to published information for microcapsules and fortified products. Although there are clear differences, all treatments demonstrate at least adequate retention of AA over the 24-month period when stored under cycling temperature conditions.

Conclusions

Key findings are:

1. Cellets® 350 is a superior base material compared to sugar spheres for the processing conditions used in this study.
2. Microcapsules prepared from Cellets® 350 were of a suitable quality when lower levels of AA loading (≤6%) and lower concentrations of hydrocolloids (0.75% to 4%) were used, but at higher AA loading levels (10%) and higher concentrations of hydrocolloids (8%) processing was not successful due to agglomeration which resulted in bed collapse.
3. Losses of AA during storage for two years were moderate (~30%) for treatments with a low loading level (1.25%) and very low (approaching zero) for treatments with a higher loading level (~6%).
4. Sodium Alginate with and without Hi-maize® provided the best overall performance with low processing losses and high retention of AA during storage.

Recommendations

It is recommended that the following research activities be undertaken:

- Incorporate microcapsules—prepared by fluidised bed processing of the materials that have performed well in this study—into food matrices representative of those used in combat ration packs.
- Monitor the performance of microencapsulated AA in the selected food matrices, including storage trials and sensory evaluation.
- Investigate the potential for protective effects when combinations of wall materials and vitamins are used.

It is also recommended that the release characteristics of the microcapsules be investigated.

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Contents

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1. Introduction

1.1 Background

Nutrition, through its effects on physical and cognitive performance, and immune status, plays a fundamental role in ensuring that Australian Defence Force (ADF) personnel are operationally ready (Forbes-Ewan, 2009). The provision of fresh food to soldiers is a priority for the ADF, however when the use of fresh food or canned equivalents is not practicable, combat ration packs (CRP) are used instead (Department of Defence (DoD), 2008a).

As CRP may be the sole food source for periods of several weeks, it is important that they provide sustenance for the soldier and allow the maintenance of a high level of nutritional status. Military recommended dietary intake (MRDI)[1] values and recommended nutritional criteria (RNC) for CRP have been established (Forbes-Ewan, 2009) to specify appropriate levels of nutrients to be consumed by the soldier and provided in CRP, respectively.

Several factors contribute to a risk that soldiers may not receive adequate nutrition while they are reliant on CRP. The storage and distribution system provides for a delay of up to three years and six months before issue to the consumer (DoD, 2009)[2]. Storage may occur in northern Australia under tropical climate conditions. During the storage period vitamin levels decrease, in some cases dramatically. The sensory acceptability of the food also declines during storage, exacerbating the fact that CRP are generally under-consumed (Booth *et al.*, 2001; Carins 2002; Carins and Tennant, 2010). In addition, some nutrients are poorly distributed across the range of components; therefore the adequacy of intake may be significantly affected by the choices of the consumer.

Consequently, soldiers may fail to meet the MRDI for particular vitamins. Of particular concern, are the naturally low levels, high loss rates and poor distribution of ascorbic acid (AA) across the range of CRP components (Probert and Bui, 2013, in preparation). AA is one of the most unstable water soluble vitamins during food processing and storage (Keijbets and Ebbenhorst-Seller, 1990; Burdurlu *et al.*, 2006; Bui and Coad, 2011). High losses from high moisture foods are not unusual, however high losses are also experienced from relatively low moisture products including chocolate, fruit grains, multivitamin tablets and microcapsules (Table 1).

[1] MRDI have been developed for a range of activity levels, age groups and for male and female ADF members (Forbes-Ewan, 2009).

[2] Patrol Ration One Man ration packs may remain in the system for three years and six months when stored in temperate climates and three years in tropical climates. Combat Ration One Man ration packs may remain in the system for two years and nine months when stored in temperate climates and two years and three months in tropical climates (DoD, 2009).

These problems may be at least partially addressed through the fortification of CRP components[3]. Australia, the USA and several other nations currently fortify CRP components with a range of vitamins. In order to ensure that sufficient fortificant to confer the intended benefit remains at the time of consumption, it is important that added vitamins are protected during processing and storage. One method of protection of fortificants is microencapsulation.

Table 1: Vitamin C (measured as % of AA) losses of dried items at various storage conditions

Losses (%)	Products	Storage conditions	References
40	Ready-to-eat cereals	Room temp/~12 mth	Steele, 1976
16	Powder fruit drinks	21 °C/day	Mehansho *et al.*, 2003
82	Potato flakes (fortified with L-ascorbic acid)	25 °C/4.3 mth	Wang *et al.*, 1992
23	Multivitamin tablet in plastic container	25 °C/6 mth/75% RH	Ottaway, 2008
8–11	Fortified freeze dried meals	37 °C/24 mth	Bui and Coad, 2011
2–7	Microcapsules	38 °C/4 days/84% RH	Uddin *et al.*, 2001
4–19	Microencapsulated AA, 6% loading (spray drying technique)	37 °C/15 mth	(*unpublished data*, Wijaya's PhD data)
80	Bran flakes (moisture= 11%)	40 °C/1 mth	Wang *et al.*, 1992
19	Nutritional beverage powder	43 °C/5 mth/50% RH	Fenton-May, 1975
8–20	Microencapsulated AA, 6% loading (spray drying technique)	48 °C/6 mth	(*unpublished data,* Wijaya's PhD data)

Note: mth = month(s)

1.2 Microencapsulation of food ingredients

Microencapsulation has been used widely in the food industry for the last six decades to protect bioactive, or functional, food components (Desai and Park, 2005; Vilstrup, 2001). The purpose is to provide a coating to protect the bioactive component when it comes into contact with oxygen, pH, light or other ingredients in the food matrix (Augustin and Hemar, 2009; Champagne and Fustier, 2007; Dziezak, 1988) and to increase the shelf life of sensitive ingredients during storage (Augustin and Hemar, 2009; Hirotsuka *et al.*, 1984). The coating can also mask unpleasant tastes without compromising the product's

[3] This is consistent with a requirement that the manufacturer of CRP components provide products capable of withstanding lengthy storage under harsh storage conditions. CRP are subject to a shelf life warranty of two years when stored at 30 °C, although this requirement is not consistently expressed in relevant documentation (DoD, 2008b, 2009).

intended taste. This property may be useful when adding functional ingredients that have an unpleasant taste.

Microencapsulation can also help to deliver nutrients or bioactive component to targeted sites in the gastrointestinal tract (de Vos *et al.,* 2009). Controlled release at targeted sites may be achieved by gradual discharge of the core materials by diffusion through the microcapsule walls or when external conditions trigger the microcapsule walls to rupture or dissolve (Chulia, 1994; Pothakamury and Barbosa-Cánovas, 1995).

Although extensively studied, there is no universally accepted procedure for the microencapsulation of bioactive food components. This may be due to differences in the chemical structure of individual bioactive components (Augustin and Hemar, 2009; Kailasapathy, 2002), the processing conditions involved in manufacture and differences in the intended end use of the product.

Microencapsulation may be achieved by many different techniques depending on the specific requirements. The most common technique, spray-drying, has been applied to the microencapsulation of AA for the fortification of combat ration pack components (Wijaya *et al.,* 2011). An alternative technique, fluidised-bed coating, results in microcapsules with different physical characteristics to those produced by spray drying. The different physical and performance characteristics of the two types of microcapsule will determine their individual suitability for the fortification a range of food matrices.

A review conducted to assess the potential suitability of commercially available, stable forms of microencapsulated vitamins for the fortification of CRP components (unpublished data), found that the available products did not meet Defence's CRP shelf life requirement of 2 years at 30 °C (DoD, 2008b).

1.3 Aims

The aims of this study were to:

- Prepare microencapsulated AA using a fluidised-bed coating technique.
- Optimise the fluidised-bed processing parameters.
- Investigate the suitability of different base and wall materials as encapsulating agents for AA. This includes consideration of processing characteristics and effectiveness in the protection of AA during storage under tropical conditions.

2. Materials and Methods

2.1 Microcapsule materials

In this study, the coating materials used in the production of microcapsules were food grade hydrocolloids. These hydrocolloids are water soluble, serve both as binders (encapsulating agents) and carriers, are used widely for microencapsulation (and more generally in the food industry) to enhance the textural properties of food. In addition, Hi-maize® has been used together with some hydrocolloids to see whether it might influence microcapsule characteristics or storage stability. Hi-maize® is high in resistant starch and has been used elsewhere in coating preparations to encapsulate the active ingredient, for example, probiotics (Kailasapathy, 2002; Rokka and Rantamäki, 2010), folic acid (Madziva *et al.,* 2006) and ascorbic acid (Wijaya *et al.,* 2011).

The base materials were sugar spheres and Cellets® 350. Sugar spheres (water soluble pellets made of sugar) and Cellets® 350 (water insoluble pellets made of microcrystalline cellulose) are listed as 'generally recognized as safe' (GRAS) (Smith and Hong-Shum, 2003). These spheres are of relatively uniform size, have excellent flow ability, are safe for consumption and have been used in the pharmaceutical industry in controlled release applications. In contrast with sugar spheres, the Cellets® 350 are inert, tasteless and more versatile in terms of end product application. The details of the coating materials and base materials (sugar spheres and Cellets® 350) are presented in Table 2.

Table 2: Food grade materials used in the preparation of microcapsules

Ingredient	Description	Manufacturer
Sugar spheres SANAQ®	Ph. Eur. / USP-NF	Pharmatrans Sanaq AG, Switzerland
Cellets® 350	Lot No: 06G0019	
Ascorbic acid food grade (99.0-100.5%)	Product code: 158000	Bronson & Jacobs Pty. Ltd, Melbourne, Australia
Gum Arabic (GA)	Luxara 3A, Product 4417	Arthur Branwell & Co Ltd, United Kingdom
Instant 449	Pregelatinised, modified waxy maize starch	Penford Australia Ltd, Melbourne, Australia
Pharmacoat®	Grade 603, Lot no: 6036174	Shin-Etsu Chemical Co., Ltd. Japan.
Sodium alginate	Product code: 180947	Sigma-Aldrich Pty Ltd, NSW, Australia
Hi-maize®1043	Unmodified high amylose maize starch	National Starch, Sydney, Australia

Fluidised-bed coating trials were conducted on the materials detailed in Table 2 using combinations and concentrations set out in Table 3. Trials 10 and 11 are duplicate trials. The materials preparation and microencapsulation processes are detailed following the description of fluidised-bed coating techniques.

Table 3: Fluidised- bed coating trials

Trial no	Core material	Combination of wall materials	
		Loading (AA %)	Wall material
1	Sugar spheres	6	Sodium alginate (0.75%)
2	Sugar spheres	6	Sodium alginate (1.5%)
3	Sugar spheres	6	Instant 449 : Maltodextrin (2:10)
4	Sugar spheres	6	Gum Arabic : Maltodextrin (1.5:10)
5	Sugar spheres	6	Gum Arabic (2.5%)
6	Sugar spheres	6	Gum Arabic (4%)
7	Sugar spheres	6	Gum Arabic (5%)
8	Sugar spheres	6	Pharmacoat® (2%)
9	Sugar spheres	6	Pharmacoat® (4%)
10	Cellets® 350	1.25	Instant 449 (2%)
11	Cellets® 350	1.25	Instant 449 (2%)
12	Cellets® 350	10	Instant 449 (2%)
13	Cellets® 350	10	Gum Arabic (4.5%)
14	Cellets® 350	6	Pharmacoat® (8%)
15	Cellets® 350	6	Pharmacoat® (4%)
16	Cellets® 350	6	Pharmacoat® : Hi-maize® 1043 (4:1) *
17	Cellets® 350	6	Sodium alginate (0.75%) *
18	Cellets® 350	6	Sodium alginate : Hi-maize® 1043 (0.75:2) *
19	Cellets® 350	6	Gum Arabic : Hi-maize® 1043 (4:1)

Notes Trials 12-14 Unsuccessful trials
Trials 16-18* Treatments with double coating
Only microcapsules using Cellets® 350 as a base material were used for storage trial

2.2 Fluidised-bed coating techniques

Microencapsulation processes are usually categorised into two groups: chemical processes and mechanical processes (Augustin and Hemar, 2009; Vilstrup, 2001). Fluidised-bed coating, a mechanical process, is commonly used to encapsulate solid core materials by introducing liquid onto porous solid material using a spray nozzle (Figure 1). The

underlying principle is that an even coating is normally achieved by spraying small droplets of a low viscosity fluid.

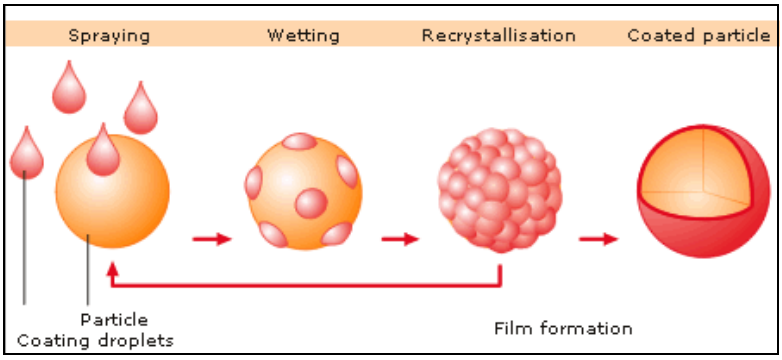
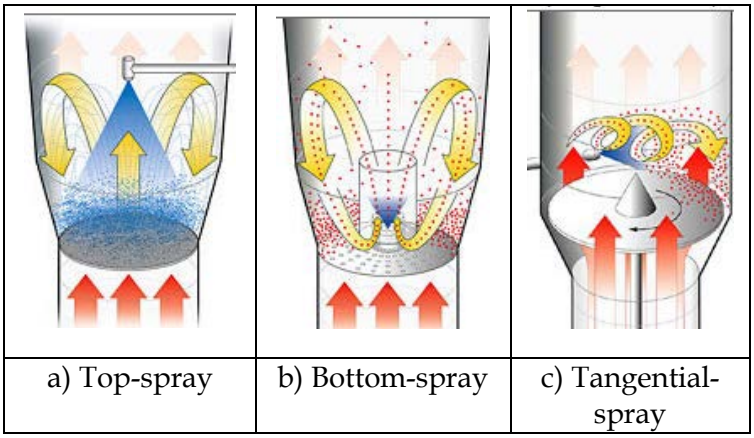


Figure 1: The formation of microcapsules by fluidised-bed coating (accessed from www.Glat.com)

There are three processing options that can be used in a fluidised-bed system: top-spray coating, bottom-spray coating and tangential-spray coating (Figure 2).



a) Top-spray	b) Bottom-spray	c) Tangential-spray

Figure 2: Three processing options in a fluidised-bed system: a) Top-spray; b) Bottom-spray; c) Tangential-spray (Accessed at www.glatt.com)

Bottom-spray coating, also known as the Wurster process, was used for this study (Figure 2b). In this, the nozzle is located at the bottom of the processing chamber and is used to spray the coating liquid vertically into an upwards directed annular air and particle flow inside the chamber. This process is commonly used in the pharmaceutical industry for active layering and for coating to modify or control drug release, and provides excellent coating uniformity and efficiency (www.Glatt.com, accessed 10 Mar 2011; Kristensen, 2005; Vertommen and Kinget, 1997; Srivastava and Mishra, 2010).

A fluidised-bed granulator, shown in Figure 3 (GPCG 1.1, Glatt, Binzen, Germany), was used for this study. All sensors and controllers were connected to a computer system. Process parameters were chosen to ensure constant inlet temperature and exhaust air temperature for the entire process. Other processing conditions including product temperature, fluid flow rate, and air flow were monitored during use (this model does not have a humidity control unit).

Figure 3: Glatt fluidised-bed unit – pilot scale

The effectiveness of this technique relies not only on the optimisation of the operational conditions (flow rate, fluidised-bed temperature, atomising pressure, fluidising air velocity, product temperature, inlet/outlet temperature) but also depends on the structure of wall materials and the combination of wall materials and the active ingredient (AA in this case).

2.3 Microencapsulation trials

There are many factors involved in optimising the fluidised-bed processing conditions: choice of base and coating (wall) materials, mass of base material, air flow rate, spray flow rate (feeding rate), atomiser pressure, inlet temperature, product temperature, partition setting and choice of distribution plate.

In all experiments, the following factors were fixed:

1. The mass of base material was 1.5 kg regardless of the type of base material. The size range was 300–500 μm in diameter.
2. The pH of the coating materials was adjusted to pH 3.5.

Solid-base materials (sugar spheres or Cellets® 350) were suspended in a fluidised-bed chamber by a jet of air. A coating fluid carrying the material to be protected, in this case AA was sprayed on the suspended solid materials at a controlled flow rate. After

spraying, the coated microcapsules were dried and then cooled before removing them from the fluidised-bed chamber for storage and analysis.

The behaviour of the materials during microencapsulation was observed. Trials that exhibited agglomeration of particles, bed collapse or other evidence of poor microencapsulation were terminated. Trials that resulted in microcapsule breakage did not proceed to the storage study.

2.3.1 Wall material preparation

The wall materials were added to deionised water (0.75–8% w/v) and mixed by magnetic stirrer for about 10 minutes. The suspension was then covered with aluminium foil and held in an incubator at 50 °C to hydrate overnight.

The solution was allowed to cool to approximately 30 °C (while stirring). AA was added in a range of concentrations up to 10% w/v. The pH was adjusted to 3.5 using sodium hydroxide (0.1 M). Other ingredients, such as Hi-maize®, were added to some mixtures. The pH and temperature were controlled to ~3.5 and <40 °C respectively, as elevated values (pH >6.0) would result in rapid losses of AA.

Food colours were added to the mixture before fluidising in order to observe the uniformity of coating during spraying. Colour changes during storage were also monitored.

2.3.2 Fluidised-bed coating process

The fluidised-bed chamber was heated to 40 °C prior to introducing the base and feed materials (containing AA and selected wall material) to the fluidised-bed chamber. Then the base material (Sugar spheres or Cellets® 350) was added to the chamber. The chamber load was allowed to equilibrate to 40 °C then the feed material was sprayed into the chamber at a flow rate of 8–10 mL/min. The chamber contents were heated, dried and then cooled and removed from the chamber.

The fluidisation air serves as a momentum carrier, and acts as the heating or cooling medium. The fluidised-bed parameters were optimised by varying the partition positions, size of air distribution plate, controlling the spray rate, controlling the air temperature, the air flow rate and pressure. The air flow, exhaust temperature, inlet air temperature and product temperature were monitored for the entire process.

2.4 Storage trials

Storage trials were used to evaluate samples from each successful microencapsulation trial. The storage trials were conducted so that samples were stored in a temperature and humidity controlled cabinet under conditions intended to approximate the diurnal variation experienced in northern Australia. The temperature was cycled between 25 and

35 °C, completing one full cycle over a 24-hour period, for up to 24 months and relative humidity (RH) was controlled at 80%. The storage profile is shown in Table 4.

Table 4: Storage profile of microencapsulated AA

Temp (°C)	**Storage time (months)**								
	Initial	1	2	3	6	9	12	18	24
25 – 35	✓	✓	✓	✓	✓	✓	✓	✓	✓

Note Only 3 storage points were analysed for AA content: 1) initial, 2) 12 month and 3) 24 month samples[4].

For each trial, six sub-samples were collected (~50 g each) at each time point: three for the control (stored at 1 °C) and three for long-term storage (25–35 °C, 80% RH). The samples were packed in paper bags inserted into an outer high barrier foil laminate pouch with a 25 µm foil layer and then vacuum sealed. The sub-samples were removed from storage at designated time points and held at 1 °C until analysis.

2.5 Analysis of microcapsules

Chemical and physical properties of microcapsules were measured by:

- Visual observations
- Spectrophotometric colour measurements
- Environmental scanning electron microscopy (ESEM) characterisation of surface morphology
- Capillary electrophoresis (CE) to determine AA content.

2.5.1 Visual observations

Samples of microcapsules stored for 0, 12 and 24 months were observed visually to detect differences in microcapsule breakage, lumpiness and adhesion between the microcapsules.

2.5.2 Spectrophotometric colour measurements

The colour of the microcapsules was measured using a HunterLab ColorQuest XE spectrophotometer (Hunter Associates Laboratory Inc., Virginia, USA) with a CQX3163 sensor and EasyMatchQC software. A small quantity of microcapsules from each treatment was placed in a 10mm glass optical cell, with the instrument set to 10° Standard

[4] The 3 main storage points (initial, 12 and 24 month) samples were selected for vitamin analysis. If the losses of AA had been high at the 12 month point, the earlier time points would have been also analysed.

Observer and D65 target energy distribution. The colour was recorded using the CIE L*a*b* colour scale.

2.5.3 ESEM observations

The outer structure of microcapsules was observed with an FEI Quanta 200 environmental scanning electron microscope (FEI, Oregon, USA) set to an accelerating voltage of 30 kV, pressure of 0.5 Torr, spot size of 5.0 nm and working distance of 10 mm. A small quantity of each microcapsule preparation was mounted on a metal stub using double sided adhesive tape. The features were viewed under various magnifications in 'low vacuum' mode.

2.5.4 Determination of AA content by CE

AA content was determined by CE using a procedure based upon those reported by Marshall *et al.,* (1995) as well as Thompson *et al.,* (1995a, 1995b). CE is often used for determination of AA content due to the quick sample preparation, small requirement for solvents and the speed of analysis.

Triplicate sub-samples of each treatment sample (~0.10 g) were mixed with 10 mL of an aqueous solution of 0.2% D,L-dithiothreitol (DTT) using a magnetic stirrer. For each solution, 1 mL was transferred to a 10 mL volumetric flask along with 0.5 mL D-*iso*-AA stock solution (1000 µg/mL) then the flask was made up to 10 mL with Milli-Q water.

All extract solutions were filtered (0.45 µm nylon filters) into CE vials before injection onto the capillary column. In order to enhance the performance of the system and prevent blockage of the capillary, the following cleaning protocol was used between sample injections: 1) rinse with filtered sodium hydroxide (0.1 M) for 2 minutes, followed by 2) Milli-Q water for 2 minutes and 3) filtered buffer (phosphate/borate pH 8.6) for another 2 minutes.

The equipment used in our study was an Applied Biosystems instrument (model 270 A-HT) with a fused-silica capillary (undeactivated, 75 µm internal diameter, Agilent Technologies). The buffer was sodium orthophosphate-sodium tetraborate (0.02 M, pH 8.6) containing sodium deoxycholate. D-erythorbic acid (D-*iso*-AA) was used as internal standard. The instrument operating conditions were: +15 kV applied voltage, 28 °C temperature setting and 254 nm for UV detection. Data analysis was performed using Shimadzu Class LC-10 software.

2.5.5 Calculations and statistics

The AA content of samples was calculated using a standard curve based on the AA: D-*iso*-AA peak ratios. Four AA standards, 25, 50, 75 and 100 µL/mL, and a blank were used to construct the linear standard curve. The curve was plotted using Microsoft Excel and calculations performed based on a linear line of best fit.

The loss in AA levels following fluidised-bed coating was calculated as:

*Percentage processing loss = 100 * (Loading level – Measured level) / Loading level*

Changes in AA levels during storage were analysed for each treatment by simple linear regression analysis (GraphPad Prism).

AA losses during storage were calculated as the difference between the initial level and the post storage level, expressed as a percentage of the initial level. Half life calculations were based on the integrated rate law for a first order reaction:

$$\log_{10}[A] = -kt + \log_{10}[A]_0$$

where, [A] is the concentration at time t, k is the rate constant and $[A]_0$ is the initial concentration. The rate constant was estimated from a plot of $\log_{10}([A]/[A]_0)$ vs t, which has a slope of $-k$. The half life, $t_{1/2}$, was calculated using the relationship:

$$t_{1/2} = \log_{10}(2)/k$$

3. Results and Discussion

3.1 Optimisation of processing conditions

3.1.1 Processing conditions

The processing conditions were optimised (Table 5) by incremental adjustments to the operating conditions to obtain free-flowing, microcapsules with an intact, uniform dried coating. Although the processing conditions were optimised, microcapsule preparation was time consuming as some manual control was necessary (especially when sugar spheres were used as the base material) to avoid agglomeration and sticking. This was necessary mainly due to a lack of automatic humidity control on the fluidised-bed dryer.

Table 5: Processing conditions of fluidised-bed coating for microencapsulation of AA

Parameter	Condition
Inlet air temp	60 ^{o}C
Airflow	60-70 m^3/h
Air atomisation pressure	1.5 bar
Product temperature	35-40 ^{o}C
Exhaust air temperature	40-45 ^{o}C
Flow rate	5-10mL/min
Partition	3
Nozzle size	0.8 mm

3.2 Comparison of base and wall materials

3.2.1 Base materials

Using the processing conditions in Table 5, successful trials were achieved in terms of coating, regardless of the base material used. However, coating of sugar spheres was less reliable and more time consuming as it was necessary to constantly adjust and monitor the flow rate and product temperature to avoid stickiness and lumpiness of the spheres. Sugar is hydrophilic and tends to become sticky when in contact with a constantly sprayed coating medium under conditions of elevated temperature. As a result, the microcapsules often agglomerated and the bed collapsed before drying was completed (Figure 4). Sugar spheres are also prone to be milled during fluidisation and subject to breakage (Figure 5).

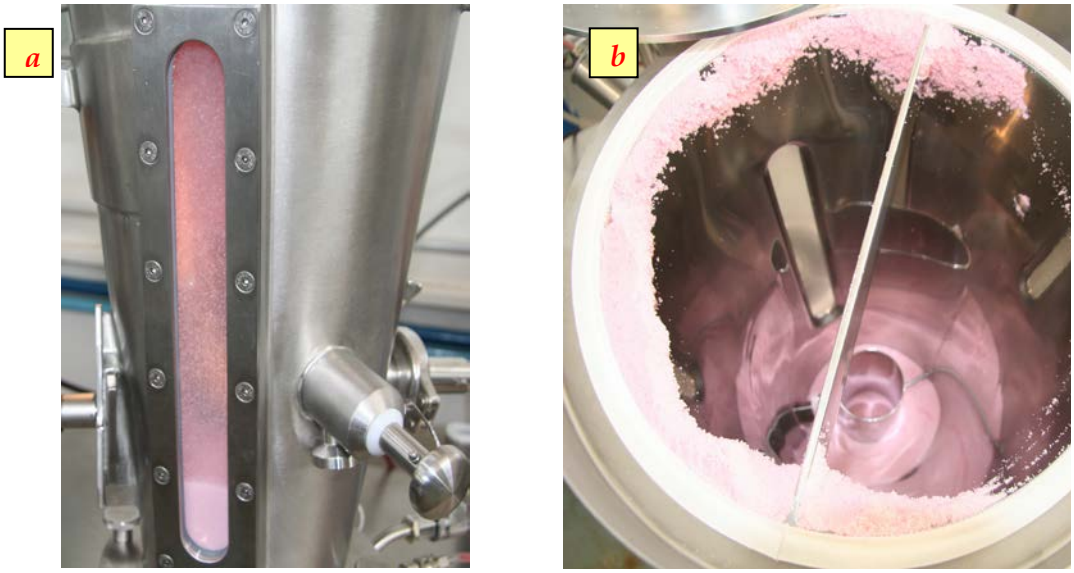


Figure 4: Photos of sugar spheres used as a base material resulted in a) product stuck to the fluidised-bed chamber; b) blockage of the nozzle inside the chamber and bed collapse

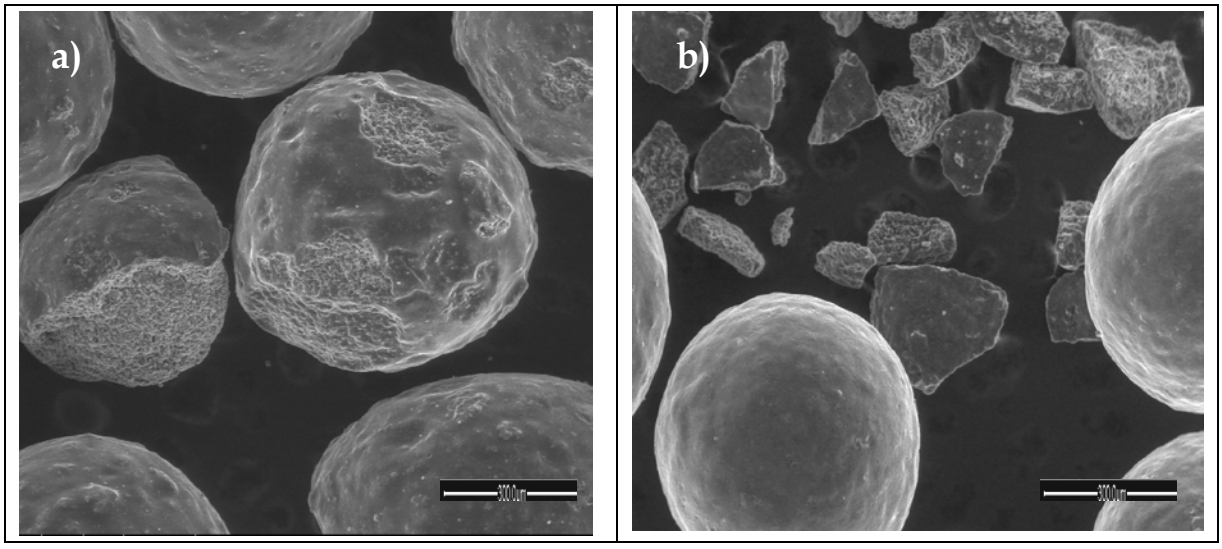


Figure 5: Breakage of sugar spheres during processing a) Gum Arabic (5%); b) Pharmacoat® (4%) (ESEM images at 100x magnification)

Although initially an electrostatic charge developed while the Cellets® 350 were heating to ~40 °C in the chamber, Cellets® 350 were much easier to handle than the sugar spheres throughout the fluidisation process. Agglomeration was a problem with some combinations of loading and wall materials, but this was less of a problem with Cellets® 350 than sugar spheres. This may be due to the high abrasion resistance of Cellets® 350 which enhanced the coating process. There was also no milling or breakage found on the final products and after storage trials for up to 24 months.

Microcapsules based on Cellets® 350 were selected for further evaluation and storage trials. Due to the processing difficulties and microcapsule breakage associated with sugar spheres, none of the microcapsules based on sugar spheres were further evaluated. A comparison of observations on the characteristics of the two base materials is provided in Table 6.

Table 6: Comparison of the characteristics of the base materials

Sugar spheres	**Cellets® 350**
Sweet	Inert, odourless and tasteless
Tacky surface during processing	Even and dense surface
Poor abrasion resistance	High abrasion resistance
Agglomerated during fluidisation	Much less likely to agglomerate
Occasionally resulted in bed collapse, leading to uneven coating	Bed collapse unlikely, even coating
Caused problems due to milling of the spheres under some conditions	Have low friability therefore did not mill or break during processing
No electrostatic charge	May develop an electrostatic charge until coated

3.2.2 Wall materials

The relatively poor overall performance of sugar spheres compared to Cellets® 350 was clearly demonstrated. Although processing was time consuming and difficult for all preparations based on sugar spheres (trials 1 to 9), some degree of success was experienced with Gum Arabic at concentrations of 2.5% to 5% on sugar spheres (trials 5 to 7). These were successfully fluidised—without bed collapse—with an AA loading of 6%. Breakage of microcapsules was a problem with all combinations of wall materials when coated onto sugar spheres.

Trials with Instant 449 (2%) and a low AA loading of 1.25% (trials 10 and 11) resulted in the formation of good quality microcapsules. Trials 12 and 13 (Table 3) indicated that Instant 449 (2%) and Gum Arabic (4.5%) coating materials prepared with a high concentration of active ingredient (10% AA loading) were unsuitable due to an intense agglomeration and bed collapse during fluidisation. This prevented the formation of dry, free flowing microcapsules.

Pharmacoat® at 8% on Cellets® 350 with an AA loading of 6% (trial 14) resulted in bed collapse, whereas at the same AA loading (6%) but a lower concentration of Pharmacoat® (4%, trial 15) fluidisation was successful. Based on visual observations, the viscosity of the more concentrated preparation was noticeably higher than that of the less concentrated preparation; this may have contributed to the bed collapse. When spray drying, higher viscosities can result in the formation of larger droplets during spraying, compromised coating of the surface of the core materials and impaired drying (Re´, 1998). A similar effect may have occurred here.

Successful fluidisation was achieved for the combinations of materials used in Trials 10, 11, 15, 16, 17, 18 and 19 (Table 3). Microcapsules from these trials were placed on storage, renamed "treatments" and relabeled from T1 to T7 respectively (Table 7) for convenience.

Table 7: Selected treatments for storage trials

Trial no	Core material	Combination of wall materials		Treatment
		Loading (AA %)	Wall material	
10	Cellets® 350	1.25	Instant 449 (2%)	T1
11	Cellets® 350	1.25	Instant 449 (2%)	T2
15	Cellets® 350	6	Pharmacoat® (4%)	T3
16	Cellets® 350	6	Pharmacoat® : Hi-maize® 1043 (4%:1%)	T4
17	Cellets® 350	6	Sodium alginate (0.75%)	T5
18	Cellets® 350	6	Sodium alginate : Hi-maize® 1043 (0.75%:2%)	T6
19	Cellets® 350	6	Gum Arabic : Hi-maize® 1043 (4%:1%)	T7

T1, T2, T3 and T7 were coated using the wall material preparation in a single layer. T4 was coated using the wall material preparation containing AA (800 mL) followed by a second coating with the wall material preparation without AA (200 mL). T5 and T6 were coated using the wall material preparation containing AA (700 mL) followed by a second coating with 0.15 M aqueous $CaCl_2$ solution (300 mL).

3.3 Analysis of microcapsules

3.3.1 Visual observations

There was no breakage of microcapsules during storage, regardless of storage time. However, there were noticeable colour changes and some darkening after 12 and 24 months.

During storage, adhesion between microcapsules developed. This was less noticeable in the control samples and most obvious for the samples stored at 25–35 °C for 24 months. The pressure required to separate the microcapsules ranged from soft to firm finger pressure. The adhesion may be due to the combination of the vacuum packed microcapsules being firmly pressed together over an extended period of time under the storage conditions used in this study.

3.3.2 Spectrophotometric colour measurements

The colour change from initial values for each treatment after 24 months storage is presented in Figure 6. The colour changes have been towards slightly decreased L* values (darkening), increased 'b*' values (more yellow, less blue) and for all except one sample slightly increased 'a*' values (more red, less green).

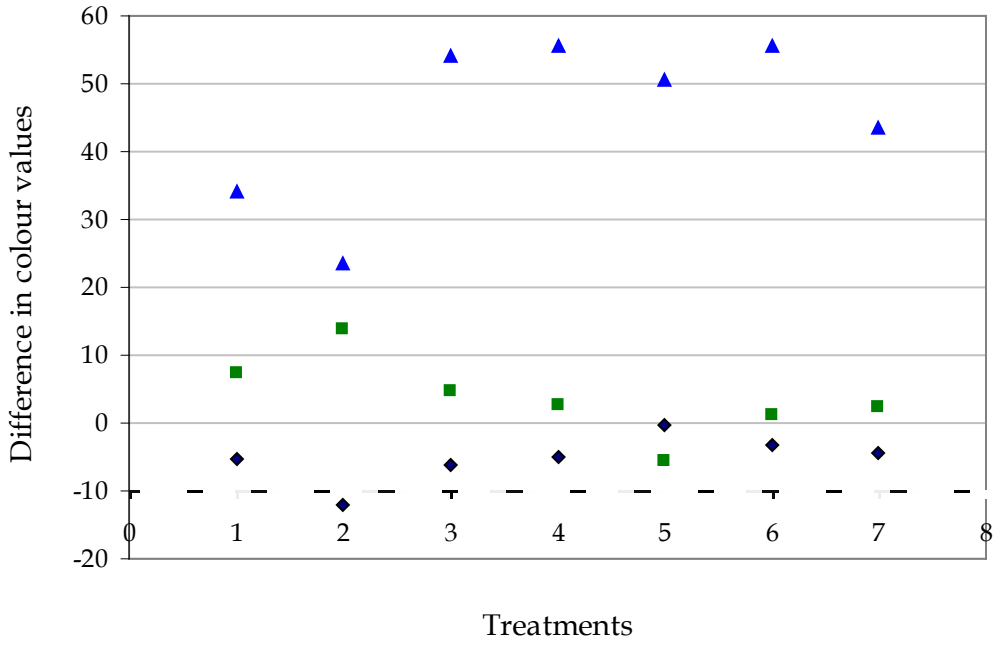


*Figure 6: Colour difference values (change from initial) ⊿L** (◆), *⊿a** (■), *and ⊿b**(▲) *for each treatment*

Notes ***L*****: Dark (0) to white (+ve);* ***a*****: Green (-ve) to red (+ve);* ***b*****: Blue (-ve) to yellow (+ve)*

3.3.3 ESEM observations

Prior to coating the Cellets® 350 particles were approximately spherical (Figure 7). Neither breakage nor milling was evident for any treatment following storage. Control and Treatment microcapsules were spherical, well-coated and free of surface defects (cracking or splitting of the wall materials) regardless of storage time. Micrographs showing the typical appearance of microcapsules are presented below (Figure 8). The full set of micrographs for all treatments is provided in Appendix A. As none of the microcapsules were broken, some were deliberately crushed to observe the thickness of the coating layer (Figure 9).

Different coating materials resulted in different characteristics on the outer surface of the microcapsules. The incorporation of resistant starch (Hi-maize®) in the coating solutions created a distinctive roughened appearance on the surface of the microcapsules which was more pronounced at the higher levels of incorporation of resistant starch. The ESEM results indicate that all treatments (T1 to T7) resulted in microcapsules with intact surfaces that are resistant to cracking and breakage.

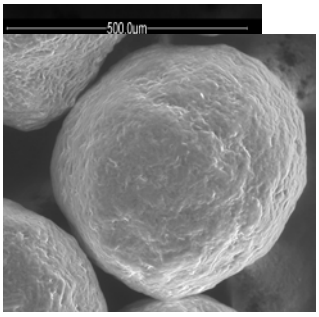


Figure 7: Uncoated Cellet® 350

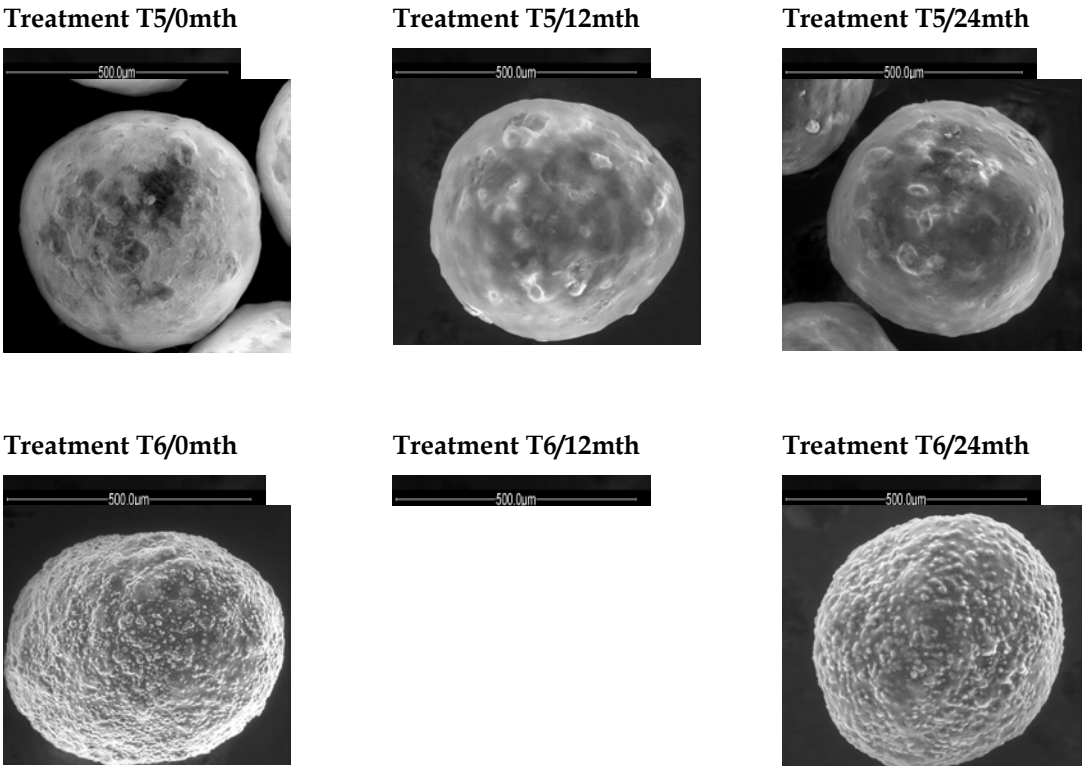


Figure 8: ESEM images of microcapsules from Treatments T5 and T6

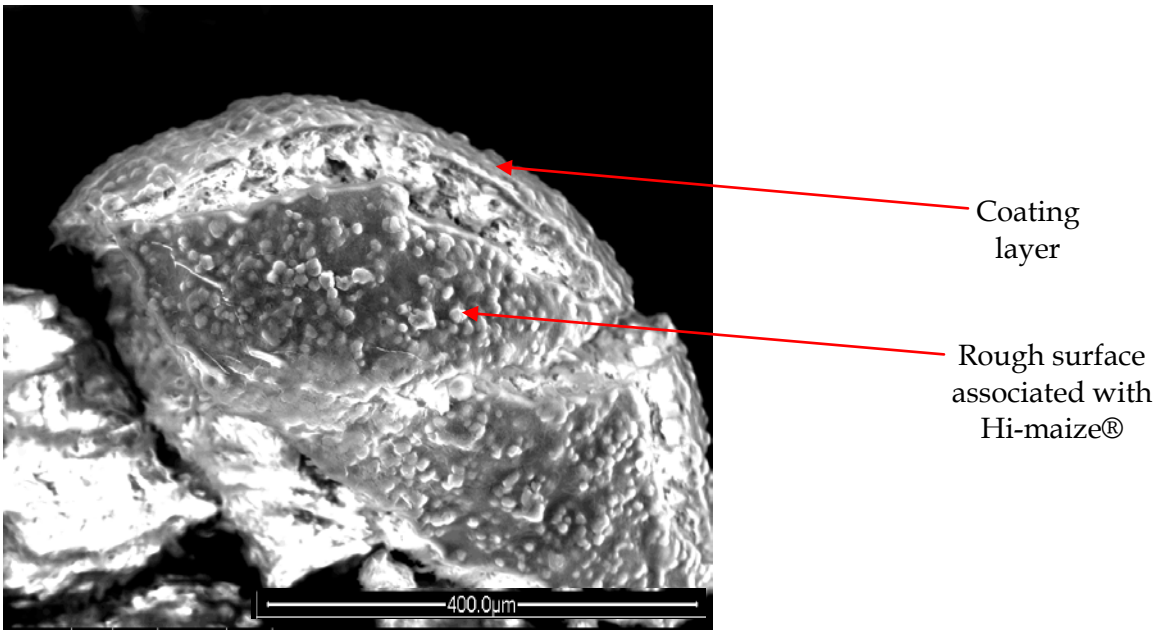


Figure 9: Microcapsule crushed to expose the coating layer (Treatment T6); (ESEM images at 400x magnification)

3.3.4 AA analysis by CE

A typical standard curve is presented in Figure 10. The standard curves were consistent over time and with high correlation coefficients (0.99 to 1.00). An example of an electropherogram is shown in Figure 11. The AA peak is clear and well separated from that of the internal standard (D-*iso* AA).

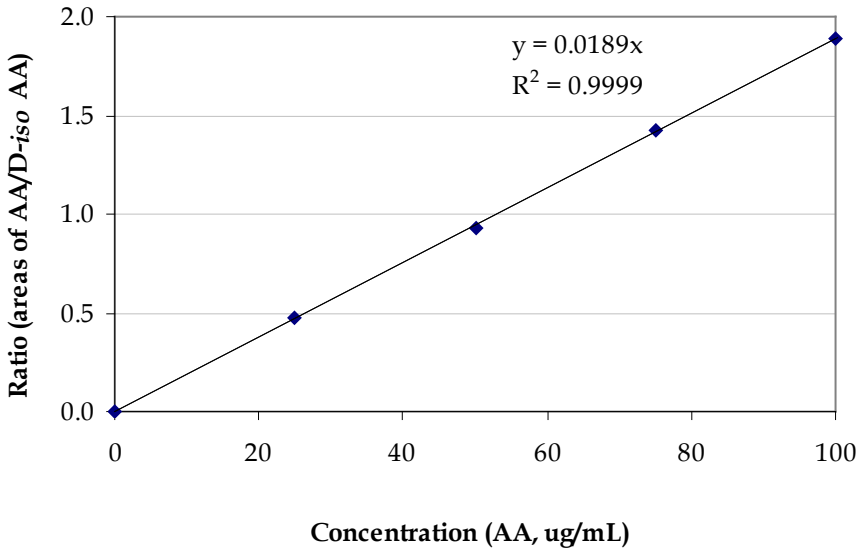


Figure 10: Standard curve prepared at concentrations of 25, 50, 75 and 100 ug/mL. Ratio represents that between areas of AA and D-iso AA. The instrument was set to zero using the buffer

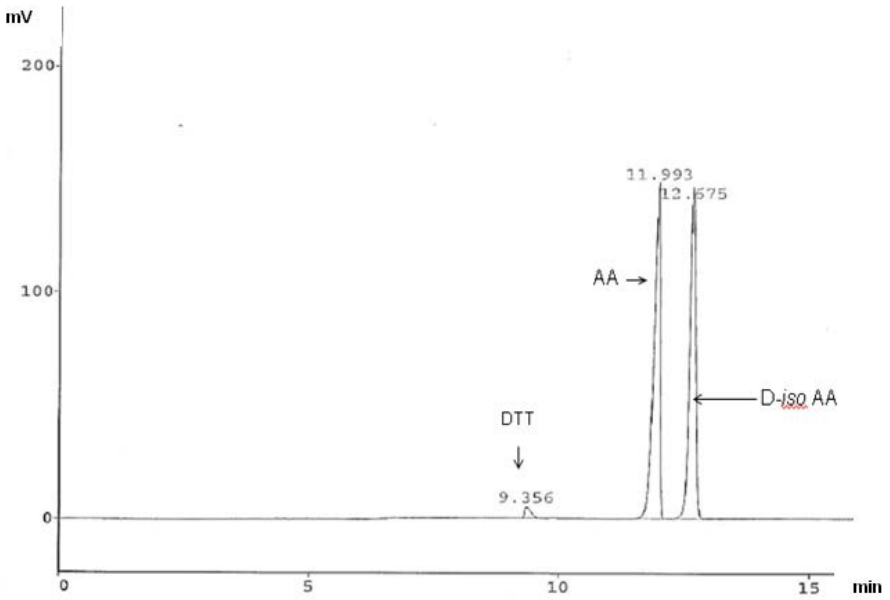


Figure 11: Electrophorogram showing separation of L-ascorbic acid from D-iso AA (Treatment T3, 12 months storage). (Vertical scale is detector response in arbitrary units, horizontal scale is elution time, in min)

3.3.5 Processing losses

The fluidised-bed coating process exposes the feed material to elevated temperatures and results in some losses of AA. The losses (the difference between the measured levels of AA in the microcapsules compared to the calculated loading levels) are shown in Table 8 expressed as percentages of the calculated loading level.

Table 8: Losses of AA during processing

Treatment	% loss	SD
T1	8.3	1.11
T2	5.8	0.46
T3	8.5	1.41
T4	9.4	1.31
T5	3.8	0.30
T6	3.4	1.10
T7	5.4	1.21

3.3.6 Storage trial results

The measured level of AA in the microcapsules following processing is referred to as the initial level in subsequent discussions regarding the outcome of the storage trials. The initial, 12-month and 24-month AA levels are presented in Table 9 and Figure 12. In some cases the standard deviation was close to zero, therefore error bars can not be shown for all data points plotted.

Table 9: Storage trial results – AA levels

Treatment	Coating medium	AA (mg/100 g)		
		Initial	12 mth	24 mth
T1	Instant 449 (2%)	1.15 ± 0.014	0.88 ± 0.004	0.77 ± 0.001
T2	Instant 449 (2%)	1.18 ± 0.006	0.88 ± 0.005	0.82 ± 0.018
T3	Pharmacoat® (4%)	5.53 ± 0.085	5.70 ± 0.056	5.80 ± 0.047
T4	Pharmacoat®:Hi-maize® (4:1)	5.51 ± 0.080	5.67 ± 0.048	5.49 ± 0.020
T5	Sodium alginate (0.75%)	5.82 ± 0.018	5.86 ± 0.034	5.93 ± 0.019
T6	Sodium alginate:Hi-maize® (0.75:2)	5.83 ± 0.067	5.85 ± 0.052	5.83 ± 0.038
T7	Gum Arabic:Hi-maize® 1043 (4:1)	5.75 ± 0.073	5.90 ± 0.049	5.74 ± 0.005

Note: n=3 for each point

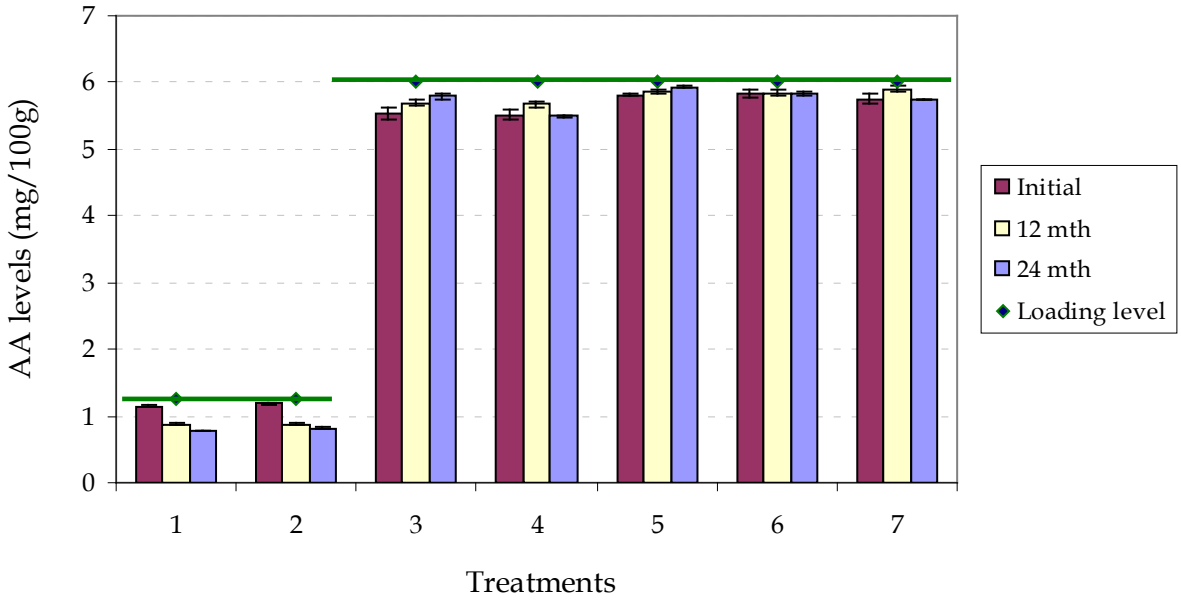


Figure 12: AA contents of microcapsules stored for up to 24 months at 25-35 °C

The data is also presented as retention of AA during storage (Figure 13). In this case the data has been normalised to 100% at time zero.

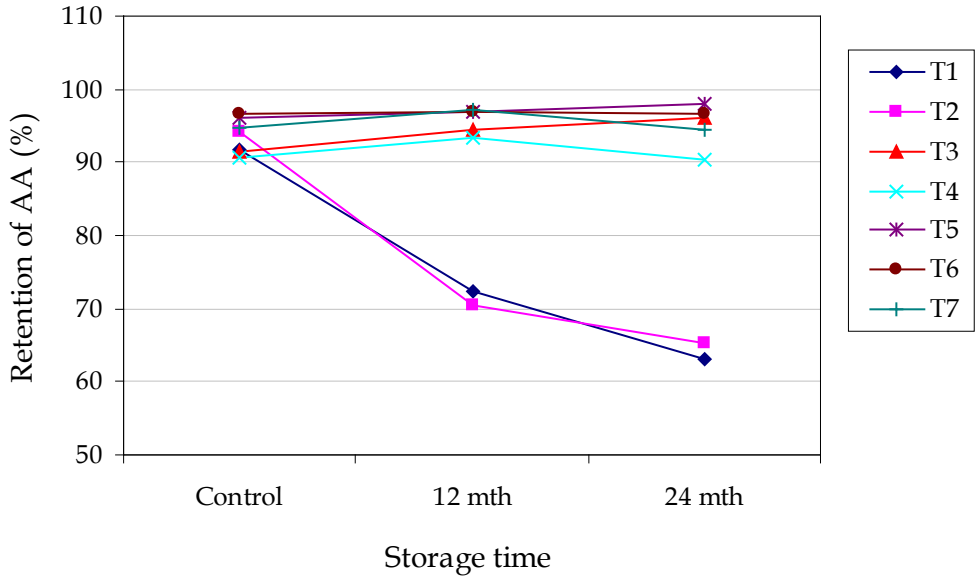


Figure 13: Relative retention of AA during storage

The data was analysed by linear regression (Table 10) and there is a significant negative association between AA level and storage time for T1 (P<0.0001) and T2 (P=0.0002). The level of AA decreased for T1 and T2 by 33% and 31%, respectively, compared to the initial levels.

Table 10: Linear regression of AA levels over 24 months for each treatment

Treatment	Slope	95% confidence interval	P value	Change over time
T1	-0.190	-0.228, -0.152	<0.0001	Highly significant
T2	-0.182	-0.242, -0.121	0.0002	Highly significant
T3	0.140	0.080, 0.200	0.0009	Highly significant
T4	-0.008	-0.109, 0.093	0.8507	Not significant
T5	0.055	0.030, 0.080	0.0012	Highly significant
T6	-0.002	-0.051, 0.048	0.9391	Not significant
T7	-0.018	-0.123, 0.088	0.6950	Not significant

There was a significant positive association between AA level and storage time for each of T3 and T5 (P~0.001 in each case). The level of AA increased over time for these treatments by 5% and 2% respectively compared to the initial levels. Although the trend is highly significant, the change is comparable to what might be expected as normal experimental

variation in analyses conducted at different time points. AA content could not increase and the mass of the Cellets® 350 base is unlikely to have decreased due to moisture loss as the product was packaged in high quality material and vacuum sealed.

For T4, T6 and T7 there was no evidence of association between AA level and storage time. The AA levels after 12 and 24 months storage were not significantly different to the initial levels. The initial AA level for T3 was not significantly different to the initial AA level for T4, however the rates of change during storage were different. The treatment with Hi-maize® (T4) remained stable overall whereas the treatment without Hi-maize® (T3) increased significantly. The same observations apply to T5 (without Hi-maize®) and T6 (with Hi-maize®). Figure 14 demonstrates these observations. Note however, that the values for T4 and T6 exhibit a peak at 12 months and lower values at the 0 and 24 month time points. The only other treatment that included Hi-maize® was T7 and this also displayed the same pattern (Figure 12). Further work would be required to clarify whether these observations are due to experimental variation and statistical anomalies or are indicative of processes actually taking place during storage. The important observation here is that the levels of ascorbic acid following storage under controlled cyclic conditions have remained relatively constant.

There is some evidence that higher loading levels may result in better retention during storage. AA loading levels of 1.25% (T1/T2) were associated with significant loss trends over 24 months. In contrast, when the AA loading level was 6.0% (T3 to T7) there was no overall loss trend over the storage period. This is clearly evident in Figure 12. Similarly, Wijaya *et al.,* (2011) investigated microencapsulation of AA using spray drying and found lower losses of AA at higher loading levels under isocratic storage conditions.

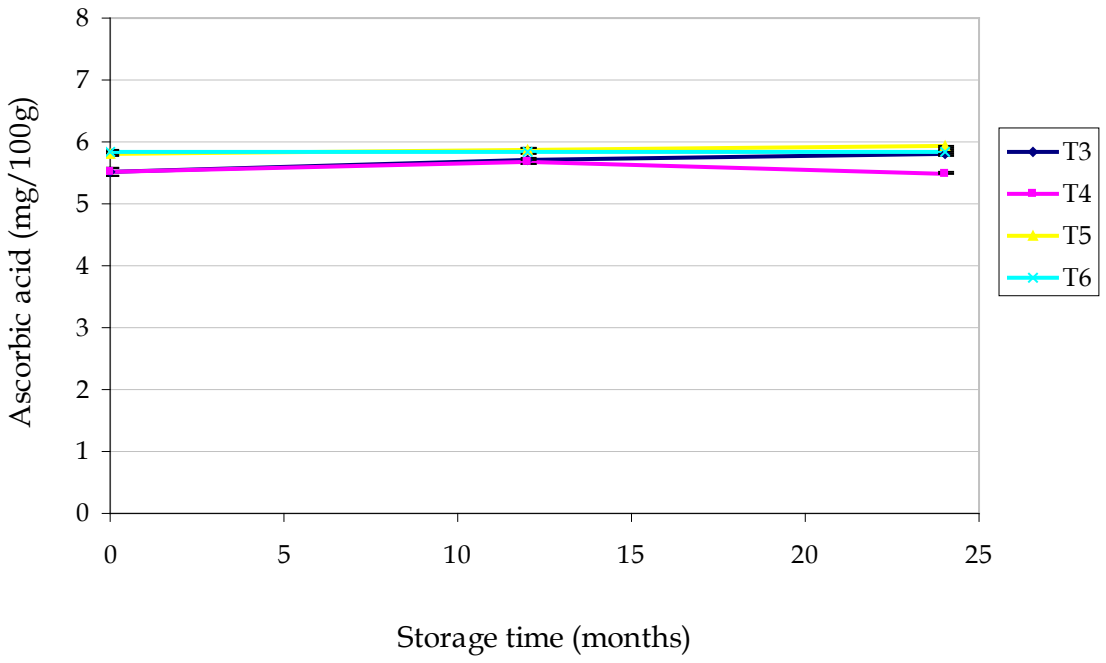


Figure 14: Comparison of treatments with (T4 and T6) and without (T3 and T5) Hi-maize®

3.3.7 Rates of loss of AA during storage

The rates of AA loss for T1 and T2 have been calculated for the 24 month period as the percentage loss per month and the half life (Table 11, Figure 15). Rate of loss calculations were not performed for treatments T3 to T7 as there were no loss trends for these treatments.

Table 11: Rate of loss of AA during 24 months storage

Treatment	Rate of loss (%/mth) [1]	Half Life $t_{1/2}$ (mth) [2]
T1	1.4	41
T2	1.3	46
T3	<1	NA
T4	<1	NA
T5	<1	NA
T6	<1	NA
T7	<1	NA

Notes 1 *The rate values are expressed in units of percentage loss compared to initial levels.*
2 *NA = Not Applicable.*

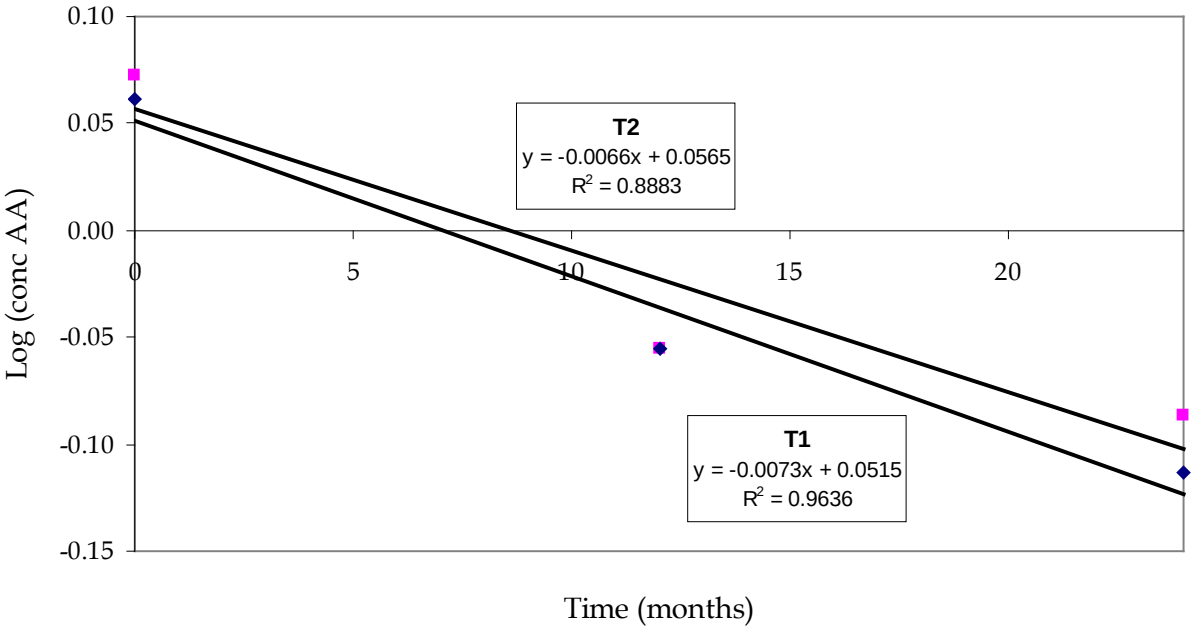


Figure 15: Log_{10} of AA levels during storage of T1 and T2 for 24 months at 25-35 °C

The results of this study indicate enhanced storage stability compared to other research results for microcapsules and fortified products (Table 1). Although there are clear differences between the higher loss treatments (T1 and T2) and the low (zero) loss treatments (T3 to T7), all treatments demonstrate at least adequate retention of AA over the 24-month period when stored under cycling temperature conditions. Very low losses (T3 to T7) are ideal and indicate a cost effective means of fortification, however higher losses of ~30% over 24 months (T1 and T2) are also manageable provided they are consistent and predictable.

The results obtained in this study demonstrate that microencapsulated AA has potential as a fortificant of food products for use in combat ration packs. The use of high-quality packaging materials and vacuum sealing may have contributed to the relatively low loss of AA. Further work is required to evaluate the storage stability of microencapsulated AA in food matrices.

4. Conclusions

The following is concluded from the work reported here:

1. Microencapsulated AA can be prepared using a range of base and coating materials. Processing conditions and choice of materials can then be optimised to minimise product loss due to agglomeration and microcapsule breakage.
2. Cellets® 350 is a superior base material compared to sugar spheres under the processing conditions used in this study. In the trials undertaken Cellets® 350 did not break, were less prone to agglomeration and were processed faster and more reliably.
3. Microcapsules prepared from Cellets® 350 were of a suitable quality when lower levels of AA loading (≤6%) and lower concentrations of hydrocolloids (0.75% to 4%) were used, but at higher AA loading levels (10%), and higher concentrations of hydrocolloids (8%), processing was not successful due to agglomeration resulting in bed collapse.
4. Analysis by ESEM revealed that the microcapsules (Cellets® 350-based) are spherical with no visible breakage following processing and long-term storage.
5. Losses of AA during storage for two years were moderate (~30%) for treatments with a low loading level (1.25%) and very low (approaching zero) for treatments with a higher loading level (~6%).
6. The presence of Hi-maize® as a co-encapsulant may improve the stability of AA during long-term storage. Further work is required to confirm this result and clarify whether there is a beneficial effect.
7. Sodium alginate, with and without Hi-maize®, provided the best overall performance with low processing losses and high retention of AA during storage.

5. Recommendations

1. Noting the results obtained to date, it is recommended that the following research activities be conducted:
   - Incorporate microcapsules—prepared by fluidised-bed processing of the materials that have performed well in this study—into food matrices representative of those used in combat ration packs.
   - Monitor the performance of microencapsulated AA in the selected food matrices, including storage trials and sensory evaluation.
   - Investigate the potential for protective effects when combinations of wall materials and vitamins are used.
2. It is also recommended that the release characteristics of the microcapsules be investigated to ascertain whether microcapsules provide fortificants with protection from food matrices while remaining susceptible to digestive processes following consumption.

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Appendix A

Table A1. ⊿L⊿a*⊿b* values from seven different treatments*

Treatments	Control	*24 mth*	Control	*24 mth*	Control	*24 mth*	Differences		
	L*	***L****	**a***	***a****	**b***	***b****	**△L***	**△a***	**△b***
1	84.2	78.9	-13.9	-6.6	20.6	54.8	-5.3	7.4	34.2
2	84.2	72.2	-13.8	0.0	20.4	44.0	-12.0	13.8	23.6
3	83.9	77.8	-14.4	-9.7	-1.2	52.8	-6.1	4.7	54.0
4	81.1	76.0	-18.4	-15.7	-5.7	50.0	-5.0	2.7	55.7
5	78.9	78.7	19.1	13.4	3.8	54.3	-0.3	-5.7	50.6
6	82.3	79.2	-13.4	-12.2	-0.3	55.2	-3.1	1.2	55.5
7	87.4	82.9	-0.1	2.3	18.4	62.0	-4.5	2.3	43.6

Notes 1 Differences on values of ⊿L⊿a*⊿b* between the control and 24mth storage samples*
2 Treatments T1 and T2 were prepared with 1.25% AA loading
3 Treatments T3 to T7 were prepared with 6% AA loading

Appendix B

Electron micrograph images of microcapsules pre- and post- storage trial

The following figures (B1 to B3) show the typical external features for the various preparations of microcapsules using ESEM. Two loading levels were used for this study: 1.25% AA loading used in Treatments T1 and T2. The remaining used 6% AA loading.

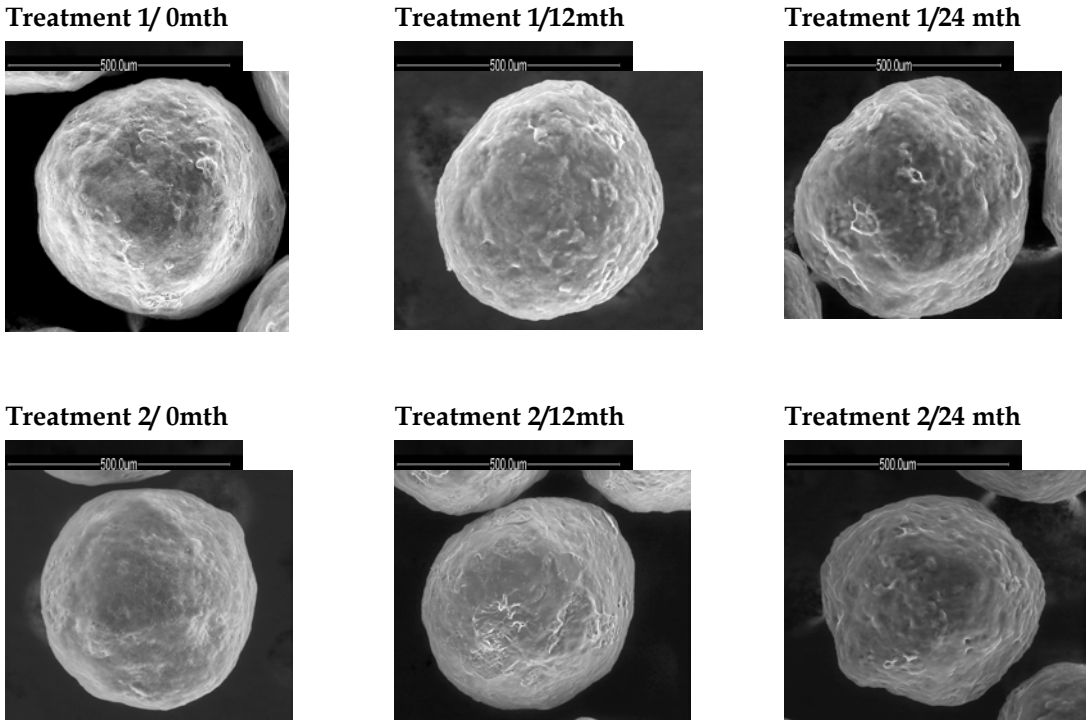

Figure B1. ESEM images of microcapsules, treatments T1 and T2

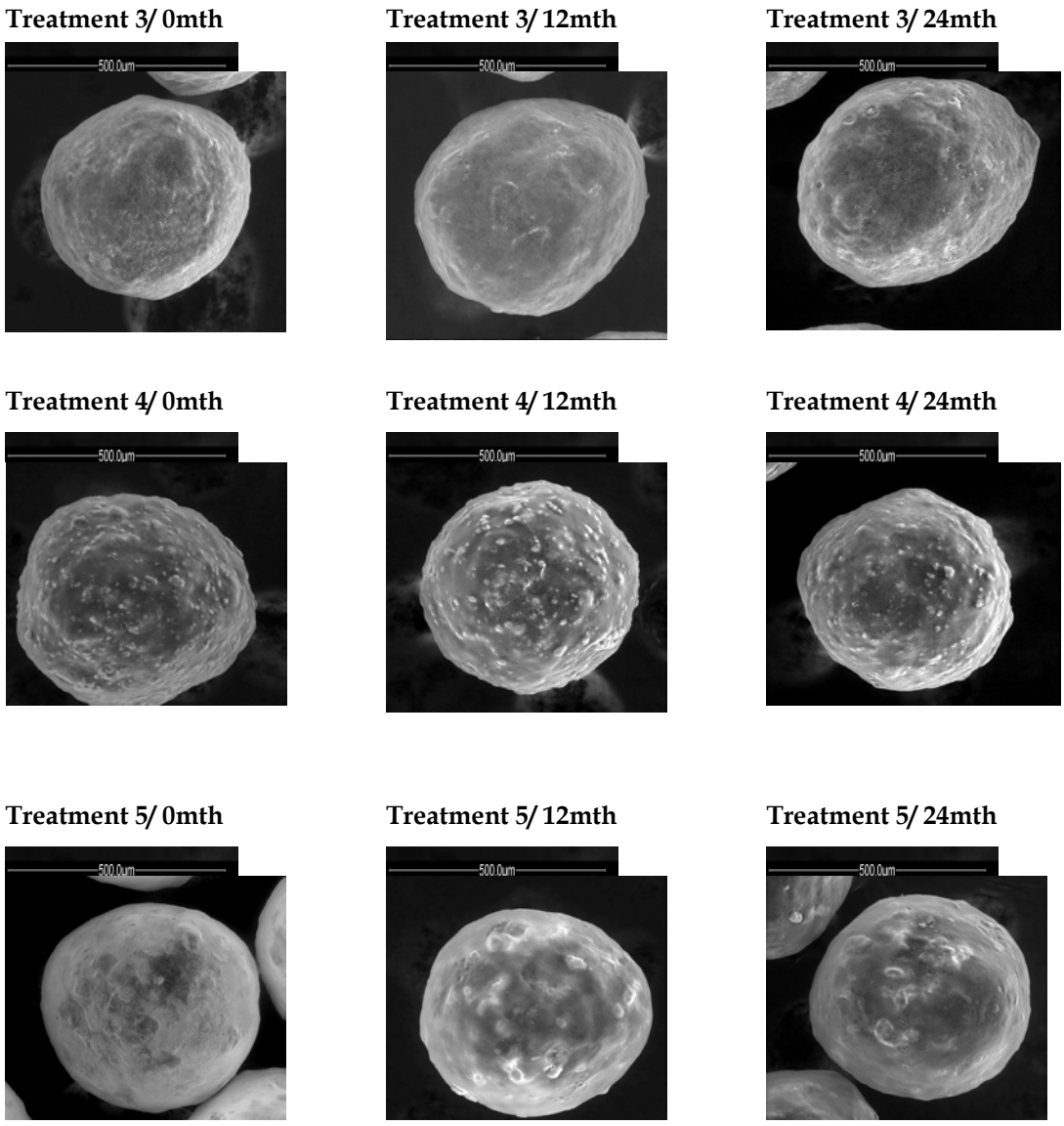

Figure B2. ESEM images of microcapsules, treatments T3, T4 and T5

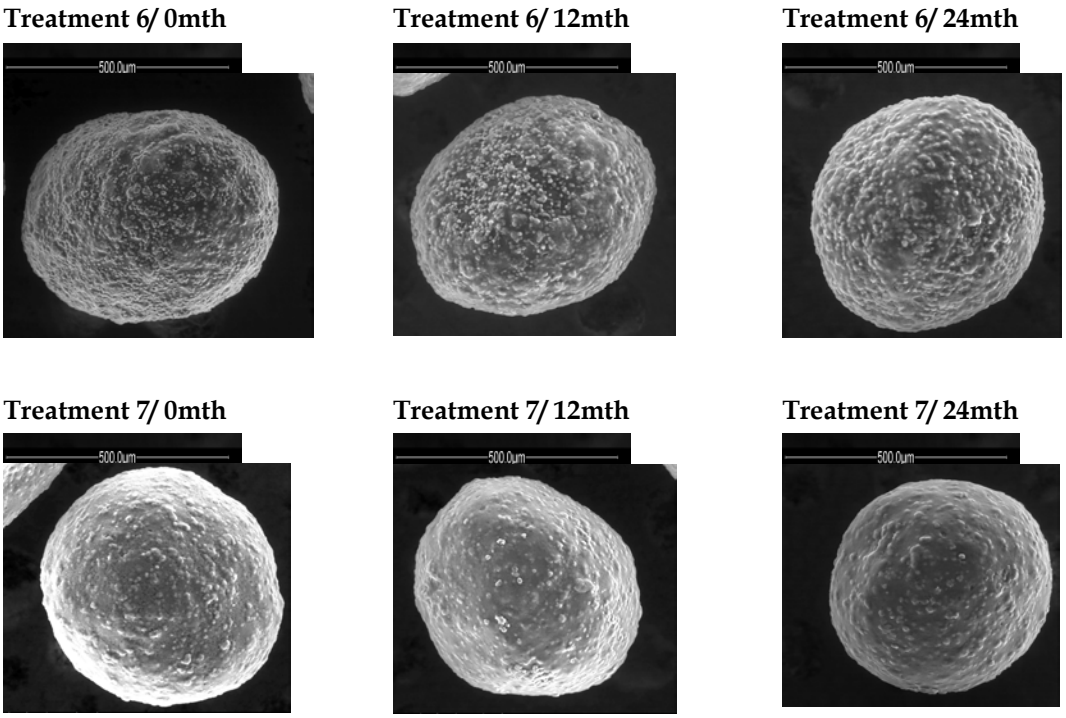

Figure B3. ESEM images of microcapsules, treatments T6 and T7

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19. ABSTRACT

This study investigates the use of microencapsulation by fluidised bed coating for the protection of ascorbic acid during long-term storage under simulated tropical conditions. Microencapsulation materials, loading levels, fluidised bed processing conditions and the results of storage trials were evaluated. Cellets® 350 is a suitable base material, whereas sugar spheres are not suitable due to agglomeration and bed collapse during processing. Sodium alginate provided the best overall performance with low processing losses and high retention of ascorbic acid during storage. The results of this study provide a basis for further research including incorporation of ascorbic acid microcapsules into food matrices using a fluidised bed coating.